

What is claimed is:

1. A re-engineered, or framework (FR)-patched immunoglobulin containing the heavy and/or light chain variable region sequences from a parent antibody, in which at least one of the compartmentalized framework sequences, defined as FR1, FR2, FR3 and FR4 are replaced, or patched by the corresponding framework sequences from the heavy and light chain immunoglobulin variable region of a different species, wherein said re-engineered immunoglobulin chain comprises framework sequences derived from at least two different sources of immunoglobulin chains, wherein said different immunoglobulin chains can be sourced from different immunoglobulins of the same species or from different immunoglobulins of different species, and such FR-patched immunoglobulin binds specifically to an antigen with affinity comparable to, or within 3-fold of, that of the parent immunoglobulin.

2. A re-engineered, or FR-patched immunoglobulin according to claim 1, in which the particular FR chosen for patching or replacing each corresponding FR in the parent immunoglobulin:

- a. exhibits the highest degree of homology, or at least 60%, to the corresponding parent FR;
- b. exhibits identical sequence homology to the corresponding parent FR at the three amino acids immediately adjacent to the flanking CDR's; and
- c. contains identical amino acid to the corresponding parent FR at positions known to be close to, or have interactions with the CDR's/antigen binding site, as evaluated by computer modeling, crystal structure, published information, or prior experience.

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3. A re-engineered, or FR-patched immunoglobulin according to claim 1, in which the particular FR chosen for patching or replacing each corresponding FR in the parent immunoglobulin:

5           a. exhibits the highest degree of homology, or at least 60%, to the corresponding parent FR;

10           b. exhibits identical sequence homology to the corresponding parent FR at the four amino acids immediately adjacent to the flanking CDR's; and

15           c. contains identical amino acid to the corresponding parent FR at positions known to be close to, or have interactions with the CDR's/antigen-binding site, as evaluated by computer modeling, crystal structure, published information, or prior experience.

15           4. A re-engineered, or FR-patched immunoglobulin according to claim (1), in which the particular FR chosen for patching or replacing each corresponding FR in the parent immunoglobulin:

20           a. exhibits the highest degree of homology, or at least 60%, to the corresponding parent FR;

25           b. exhibits the highest degree of sequence homology to the corresponding parent FR, preferably 100%, or contains conservatively similar amino acids, such as, gly, ala; val, ile, leu; asp, glu; asn, gln; ser, thr; lys, arg, and phe, tyr, at the three amino acids immediately adjacent to the flanking CDR's; and

30           c. contains identical, or conservatively similar amino acids (as listed in claim 4b) to the corresponding parent FR at positions known to be close to, or have interactions with the CDR's/antigen binding site, as evaluated by computer modeling, crystal structure, published information, or prior experience.

*Now  
lost*

*Ab A3 10 Lys*

*1st 4th 5th 6th*

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5. A re-engineered, or FR-patched immunoglobulin according to claim 1, in which the particular FR chosen for patching each corresponding FR in the parent immunoglobulin:

5           a. exhibits the highest degree of homology, or at least 60%, to the corresponding parent FR;

10          b. exhibits the highest degree of sequence homology to the corresponding parent FR, preferably 100%, or contains conservatively similar amino acids (as listed in claim 4b) at the four amino acids immediately adjacent to the flanking CDR's; and

15          c. contains identical, or conservatively similar amino acids (as listed in claim 4b) to the corresponding parent FR at positions known to be close to, or have interactions with the CDR's/antigen binding site, as evaluated by computer modeling, crystal structure, published information, or prior experience.

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25          6. A re-engineered, or FR-patched immunoglobulin according to claim 1, 2, 3, 4 and 5 containing the heavy and/or light chain variable region sequences from a parent antibody, in which the particular FR chosen for patching each corresponding FR in the parent immunoglobulin comprises re-introduced amino acids from the parent immunoglobulin framework outside the Kabat and Chothia CDRs, wherein the back mutated amino acids replace corresponding amino acids in the patching FR, which is the particular FR derived from a different source used for patching, or that replaces the original FR of, the parent immunoglobulin, and each of said back mutated amino acids:

30          a. is adjacent to a CDR in the donor immunoglobulin sequence, or  
              b. contains an atom within a distance of 4 Å of a CDR in said re-engineered immunoglobulin

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7. A re-engineered, or FR-patched immunoglobulin according to claim 1, 2, 3, 4 and 5 containing the heavy and/or light chain variable region sequences from a parent antibody, in which the particular FR chosen for patching each corresponding FR in the parent immunoglobulin comprises re-introduced amino acids from the parent immunoglobulin framework outside the Kabat and Chothia CDRs, wherein the back mutated amino acids replace corresponding amino acids in the patching FR, which is the particular FR derived from a different source used for patching, or that replaces the original FR of, the parent immunoglobulin, and each of said back mutated amino acids:

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a. is adjacent to a CDR in the donor immunoglobulin sequence, or  
b. contains an atom within a distance of 5 Å of a CDR in said re-engineered immunoglobulin.

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8. A re-engineered, or FR-patched immunoglobulin according to claim 1, 2, 3, 4 and 5 containing the heavy and/or light chain variable region sequences from a parent antibody, in which the particular FR chosen for patching each corresponding FR in the parent immunoglobulin comprises re-introduced amino acids from the parent immunoglobulin framework outside the Kabat and Chothia CDRs, wherein the back mutated amino acids replace corresponding amino acids in the patching FR, which is the particular FR derived from a different source used for patching, or that replaces the original FR of, the parent immunoglobulin, and each of said back mutated amino acids:

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a. is adjacent to a CDR in the donor immunoglobulin sequence, or  
b. contains an atom within a distance of 6 Å of a CDR in said re-engineered immunoglobulin.

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9. A re-engineered, or FR-patched immunoglobulin according to claim 1, 2, 3, 4 and 5 containing the heavy and/or light chain variable region sequences from a parent antibody, in which the particular FR chosen

for patching each corresponding FR in the parent immunoglobulin comprises re-introduced amino acids from the parent immunoglobulin framework outside the Kabat and Chothia CDRs, wherein the back mutated amino acids replace corresponding amino acids in the patching FR, which is the particular FR derived from a different source used for patching, or that replaces the original FR of, the parent immunoglobulin, and each of said back mutated amino acids:

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- a. is adjacent to a CDR in the donor immunoglobulin sequence, or
- b. is capable of interacting with amino acids in the CDRs, or
- c. is typical at its position for the species of the particular FR chosen for the patching, and the replaced amino acid in the said FR is rare at its position for the species from where the FR is derived.

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10. A re-engineered, or FR-patched immunoglobulin according to claim 1, 2, 3, 4, 5, 6, 7, 8, and 9, which specifically binds to an antigen with an affinity of between  $10^7 \text{ M}^{-1}$  and  $10^{11} \text{ M}^{-1}$ .

11. A re-engineered, or FR-patched immunoglobulin according to claim 1, 2, 3, 4, 5, 6, 7, 8, and 9, which specifically binds to an antigen with an affinity of between  $10^8 \text{ M}^{-1}$  and  $10^{10} \text{ M}^{-1}$ .

12. A re-engineered, or FR-patched immunoglobulin according to claim 1, 2, 3, 4, 5, 6, 7, 8, and 9 which is substantially pure.

13. A pharmaceutical composition comprising a re-engineered, or FR-patched immunoglobulin according to claim 1, 2, 3, 4, 5, 6, 7, 8, and 9 in a pharmaceutically acceptable carrier.

14. A method of constructing a re-engineered, or FR-patched immunoglobulin according to claim 1, 2, 3, 4, 5, 6, 7, 8, and 9 that will reduce the percentage of amino acid sequences derived from the parent immunoglobulin.

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15. A method of constructing a re-engineered, or FR-patched immunoglobulin according to claim 1, 2, 3, 4, 5, 6, 7, 8, and 9 that will reduce the immunogenicity of the re-engineered, or FR-patched immunoglobulin, when used in the intended species (for example human), compared to that of the parent immunoglobulin.

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16. A re-engineered immunoglobulin of claim 1, designated hpRFB4.

10 17. A re-engineered immunoglobulin of claim 1, designated hp1F5.

18. A composition comprising the re-engineered immunoglobin of claim 16, or 17.

15 19. A pharmaceutical composition comprising the re-engineered immunoglobin of claim 16, or 17.

20. A method for treating a subject with a cancer which over expresses CD22 comprising administering to the subject an effective amount of a re-engineered immunoglobulin of claim 16.

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21. The method of claim 20, where the cancer is Non-Hodgkin's lymphoma or rheumatoid arthritis.

25 22. A method for treating a subject with a cancer which over expresses CD20 comprising administering to the subject an effective amount of a re-engineered immunoglobulin of claim 17.

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23. The method of claim 22, where the cancer is Non-Hodgkin's lymphoma or rheumatoid arthritis.

24. A method for treating a subject with a cancer which over expresses an antigen which causes the cancer comprising administering to the

subject with an effective amount of a re-engineered immunoglobulin which is capable of binding to said antigen according to claim 1.

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